Identification and Computational Modeling of Novel Multidrug Resistance-Associated Protein-3 (MRP3) Inhibitors in Rat and Human Hepatocytes

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ABSTRACT

Over 400 transporters have been identified which contribute to membrane transport of endogenous substrates and xenobiotics. A significant number of preclinical and clinical studies pointed out that Multidrug Resistance-associated Protein (MRP; ABCC gene family) mediated efflux transport plays an important role in the systemic and tissue exposure profiles of many drugs and their metabolites and of endogenous compounds, like bile acids[1]. A problem associated with the MRP subfamily is that the exact role of the various isoforms in drug disposition is relatively hard to study, at least partly due to lack of potent and selective MRP inhibitors. The purpose of this study was to identify Mrp3/MRP3 inhibitors in rat and human hepatocytes in suspension, using the oil spin method[2]. To identify inhibitors, the Spectrum collection (Microsource Disc. Syst. Inc.) compound library (n=2000) and a selection of compounds from the Janssen corporate compound collection were screened for possible Mrp3/MRP3 inhibitors. For rat hepatocytes, 5(6)-carboxy-2',7'-dichlorofluorescein diacetate (CDFDA) was used as a model-(pro)substrate for Mrp3-mediated efflux, while for human hepatocytes acetaminophen glucuronide is used as a substrate for MRP3-mediated efflux. A naïve Bayesian model in Pipeline Pilot was constructed to elucidate important physicochemical and structural features of drugs to be selected for testing as an Mrp3/MRP3 inhibitor. **To date, 57 Mrp3 inhibitors were identified in rat hepatocytes**. These hits are likely to be primarily Mrp3/MRP3 inhibitors, i.e. in view of the affinity profile of the substrate used as well as the predominant expression of the Mrp3/MRP3 isoform (as opposed to other Mrp/MRP isoforms) at the sinusoidal membrane. At the same time, the (remaining) canalicular membrane domain in suspended rat hepatocytes accounts for only 10-15% of the total cell membrane area, indicating a limited role for Mrp2/MRP2 in this *in vitro* assay[3]. An *in vitro* assay for the identification of MRP3 inhibition in human hepatocytes is currently being developed. **In conclusion**, new and potent inhibitors of Mrp3 mediated efflux were identified by using rat hepatocytes in suspension, using an oil spin-based assay linked to fluorescence spectroscopy or LC-MS/MS. Naïve Bayesian models were elaborated to elucidate important physicochemical and structural features which an Mrp3/MRP3 inhibitor should contain. Our findings will aid in elucidating important cross-species differences in ligand affinity for Mrp3/MRP3.

OBJECTIVES

- To identify selective Mrp3 inhibitors by applying the oil spin method in rat hepatocytes in suspension.
- To develop an in vitro assay for the identification of MRP3 inhibitors in suspended human hepatocytes.
- To elucidate key physicochemical and structural features of Mrp3/MRP3 inhibitors by constructing a naïve Bayesian model.

METHODS

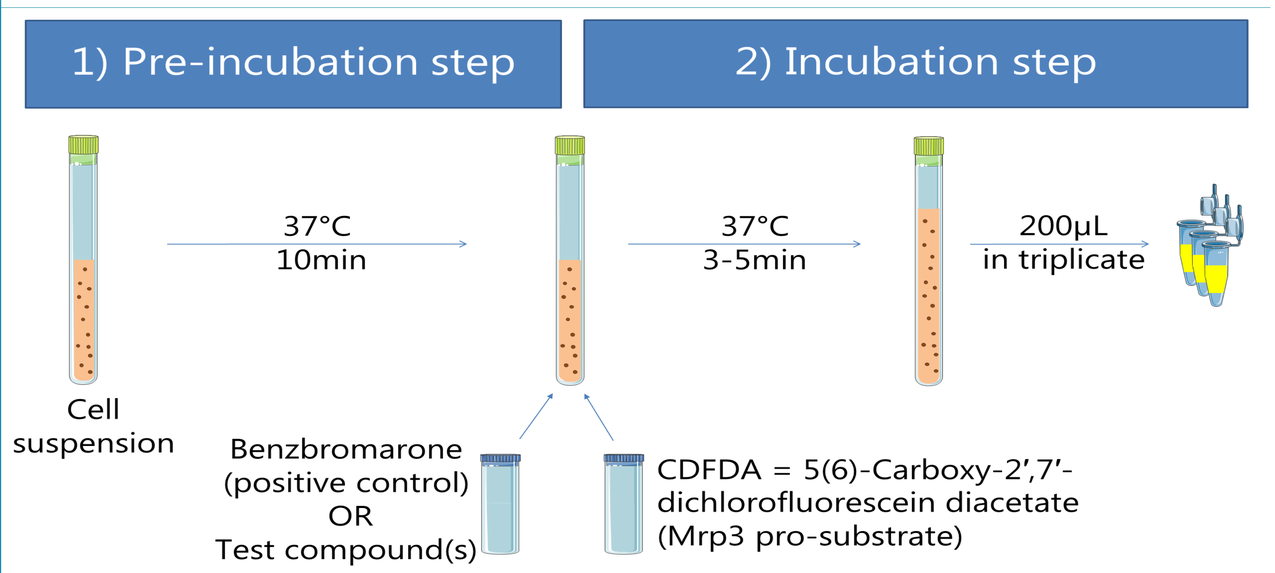

Figure 1. Optimized assay used to screen for Mrp3 inhibitors in rat hepatocytes in suspension. The Spectrum Collection (MSDiscovery), in house compounds and a selection of compounds from the Janssen corporate compound collection were used as compound libraries. In total 1584 compounds were screened.

RESULTS

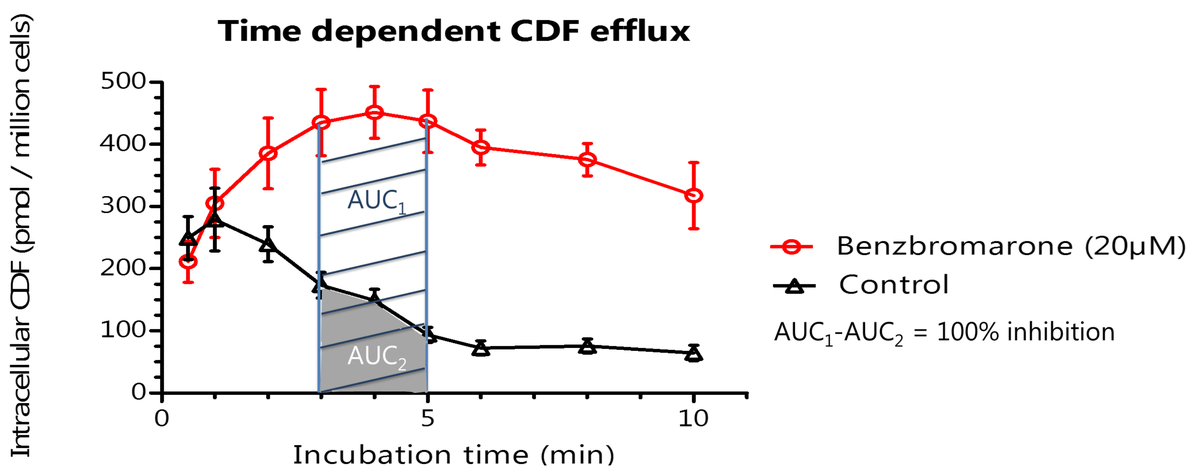

Figure 2. Time dependent profiles for intracellular CDF levels in suspended rat hepatocytes. Points are mean (±SD) CDF levels (n=3) in the absence or presence of the known Mrp inhibitor benzbromarone. The control incubation illustrates a rapid decrease due to Mrp3-mediated efflux of CDF. In the presence of benzbromarone, formation exceeds efflux, resulting in much higher levels. Based on these profiles, 3 min and 5 min were selected as time points for subsequent screening. The area under the curve (AUC) of the control condition (AUC2) is subtracted from AUC1 (benzbromarone condition) to define '100% inhibition'.

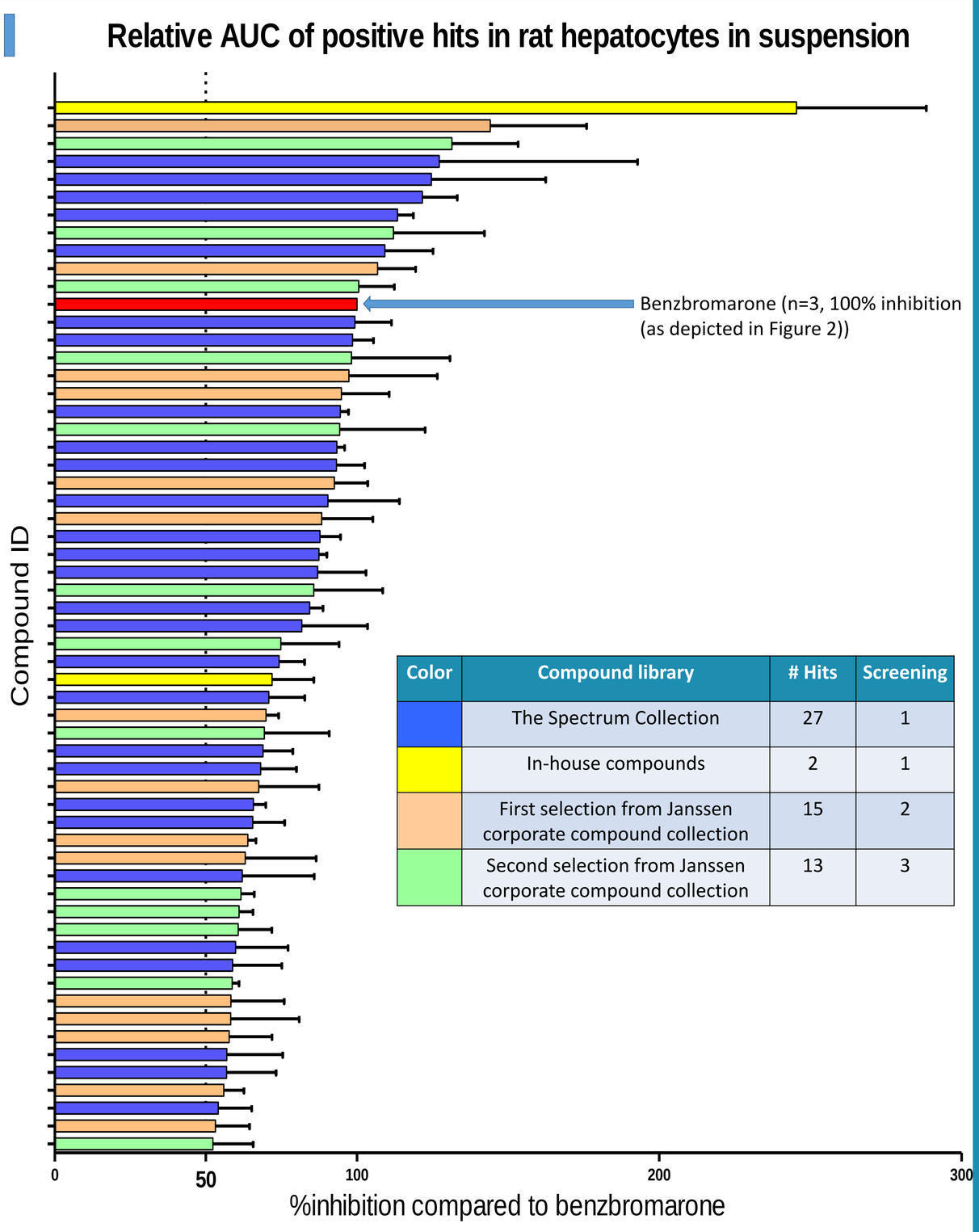

Figure 3. Overview of all compounds showing at least 50% inhibition compared to benzbromarone. Based on 3 and 5 min intracellular CDF levels, mean AUC (±SEM) values were calculated and expressed as % of the AUC difference obtained for benzbromarone (100%)(red). A 50% cut-off was chosen to distinguish between non-inhibitors and inhibitors. After screening 1444 compounds of The Spectrum Collection library and in-house compounds (Screening 1), a hit rate of 2.0% (29 hits (blue+yellow) out of 1444 compounds screened) was observed and a naive Bayesian model was constructed in Pipeline Pilot. 50 compounds were selected out of Janssen corporate compound collection based on their chemical structures as being possible Mrp3 inhibitors based on this exploratory model. This second screening resulted in a hit rate of 30% (15 hits (orange) out of 50 compounds screened). A third screening of 90 compounds resulted in a hit rate of 14.4 % (13 hits (green) out of 90 compounds screened). The total hit rate was 3.6% (57 hits out of 1584 compounds screened).

CONCLUSIONS

- Several potent Mrp3 inhibitors were identified using rat hepatocytes in suspension in this **optimized *in vitro* assay**.
- Human hepatocytes are currently used with acetaminophen glucuronide as substrate which will allow to elucidate **cross-species differences** for Mrp3/MRP3 inhibition.
- **Naive Bayesian modeling** will enable *in silico* screening of larger libraries and *in vitro* confirmation for more and potent Mrp3/MRP3 inhibitors.

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ACKNOWLEDGMENTS

This work was supported by Janssen Research & Development and a research grant from the agency for Innovation by Science and Technology in Flanders, Belgium (N° IWT150724)

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